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TITLE:	JOURNAL OF ORTHOPAEDIC RESEARCH : OFFICIAL PUBLICATION OF THE ORTHOPAEDIC RESEARCH SOCIETY
PUBLISHER/PLACE:	Raven Press New York Ny
VOLUME/ISSUE/PAGES:	1996 Mar;14(2):311-5 311-5
DATE:	1996
AUTHOR OF ARTICLE:	Alman BA; Greel DA; Wolfe HJ
TITLE OF ARTICLE:	Activating mutations of Gs protein in monostotic f
ISSN:	0736-0266
OTHER NOS/LETTERS:	Library reports holding volume or year 8404726 8648511
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Journal of Orthopaedic Research
14:311-315 The Journal of Bone and Joint Surgery, Inc.

Activating Mutations of Gs Protein in Monostotic Fibrous Lesions of Bone

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Summary: Activating mutations of the alpha chain of the heterotrimeric signal transducer Gs disrupt the inherent guanosine triphosphatase activity of the alpha chain, stimulate adenylyl cyclase, and can result in independent cell proliferation. Such mutations are identified in a number of endocrine disorders, including McCune-Albright syndrome, which is a triad of endocrinopathy, *café au lait* spots, and polyostotic fibrous dysplasia. The mutation in this syndrome is a missense point mutation in exon 8 that results in the substitution of either histidine or cysteine for arginine at position 201. Monostotic fibrous dysplasia is a nonhereditary isolated bone lesion. Other isolated bone lesions that share some cytologic and clinical similarities to fibrous dysplasia are osteofibrous dysplasia and aggressive fibromatosis involving bone. Four cases of monostotic fibrous dysplasia, four cases of aggressive fibromatosis involving bone, and one case of osteofibrous dysplasia were studied to determine if a mutation was present in exon 8 of the alpha chain of Gs. A missense mutation was present in all of the fibrous dysplasias. The other fibrous lesions and uninvolved tissue did not contain a mutation. Somatic activating mutations of Gs differentiate fibrous dysplasia from the other lesions and may be responsible for the loss of control of local proliferation and growth factor expression.

G proteins are signal transducers. Gs is a stimulatory G protein, which exists as a heterotrimer. Its alpha chain dissociates when an appropriate receptor is activated, stimulating adenylyl cyclase and the production of cyclic adenosine monophosphate (cAMP). The alpha subunit has inherent guanosine triphosphatase activity, limiting its stimulatory ability. A mutation in the alpha chain, which disrupts the guanosine triphosphatase activity, will activate G protein (8). Such an activating mutation is present in several endocrine disorders. One such disorder is McCune-Albright syndrome (16).

McCune-Albright syndrome is a triad of polyostotic fibrous dysplasia, *café au lait* spots, and endocrinopathy. Mutations have been identified in all of the affected tissues in McCune-Albright syndrome, including the abnormal endocrine tissue, the skin lesions, and the bone lesions. The identified mutations are missense point mutations within exon 8 that result in a substitution of histidine or cysteine for arginine at amino acid 201 (9,12,13,16).

Monostotic fibrous dysplasia is an isolated bone lesion that occurs in individuals without the endocrine or dermatologic manifestations of McCune-Albright syndrome. The lesions generally do not follow as debilitating a clinical course as in polyostotic disease (15). Histologically, however, fibrous dysplasia in McCune-Albright syndrome is identical to monostotic fibrous dysplasia, consisting of fibrocyte-like cells that directly produce spicules of immature bone (Fig. 1A) (6). Several other lesions share some cytologic and clinical characteristics with fibrous dysplasia. Aggressive fibromatosis involving bone (desmoplastic fibroma or extra-abdominal desmoid) is a lesion that is composed of benign appearing fibrocytes in a proliferative pattern; however, only rarely does the lesion form bone (Fig. 1B). As in fibrous dysplasia, the lesion causes local changes in bone, often recurs after excision, and increases in size during periods when circulating levels of sex steroid hormones are high (1). Fibrocyte-like cells from fibrous dysplasia and aggressive fibromatosis express several of the same proteins such as platelet-derived growth factor, estrogen and progesterone receptors, and the bone morphogenetic proteins (2,3,7,17). Osteofibrous dysplasia (Campanacci lesion) has a histologic appearance similar to that of fibrous dysplasia, but the bone is formed by osteocytes rather than directly from the fibrocyte-like cells (Fig. 1C). Some investigators think that it may be a variant of fibrous dysplasia, while others think that it may be more aggressive than monostotic fibrous dysplasia and of a different etiology (4,5,14).

The purpose of this study was to determine if an

Received May 22, 1995; accepted September 28, 1995.
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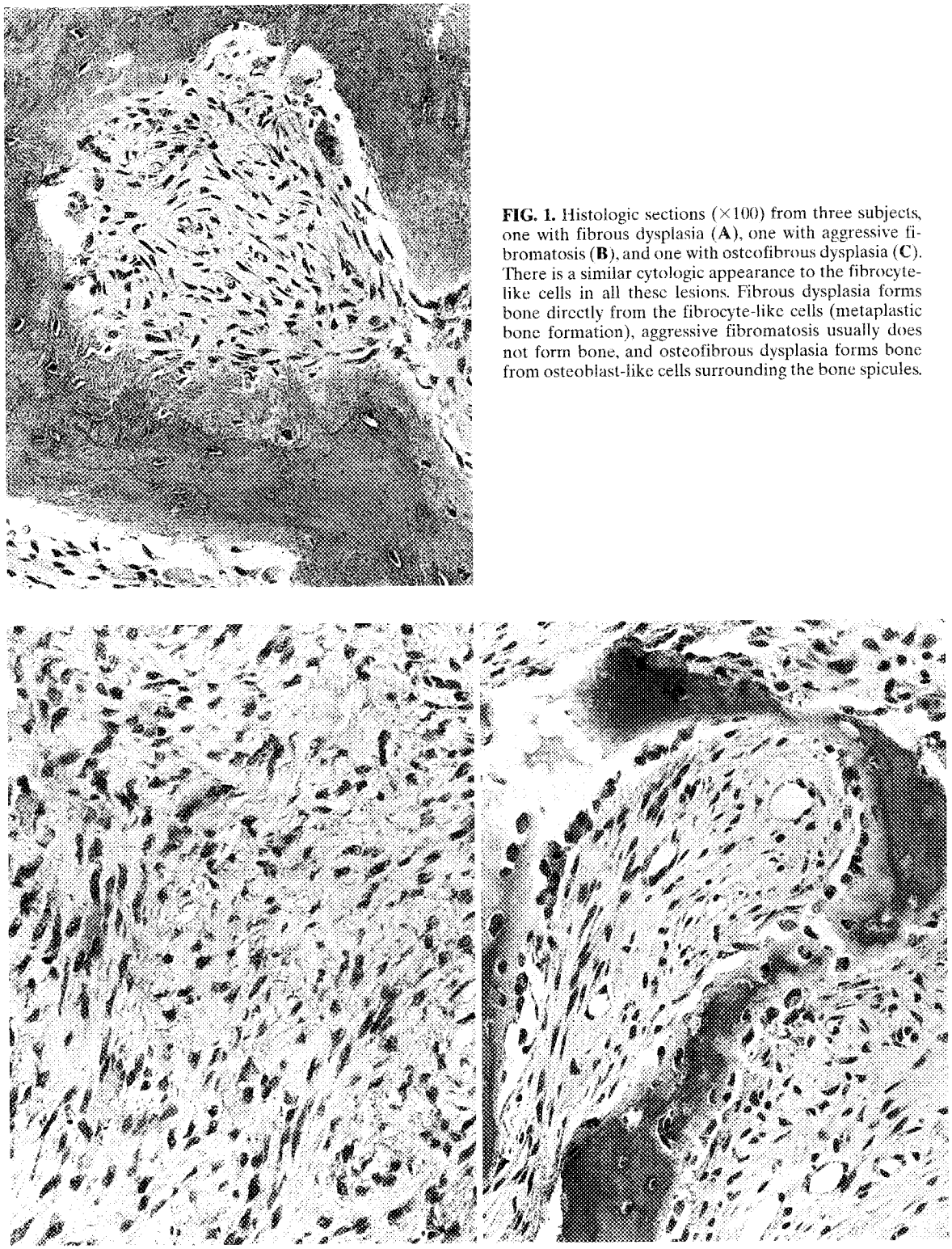

FIG. 1. Histologic sections (×100) from three subjects, one with fibrous dysplasia (**A**), one with aggressive fibromatosis (**B**), and one with osteofibrous dysplasia (**C**). There is a similar cytologic appearance to the fibrocyte-like cells in all these lesions. Fibrous dysplasia forms bone directly from the fibrocyte-like cells (metaplastic bone formation), aggressive fibromatosis usually does not form bone, and osteofibrous dysplasia forms bone from osteoblast-like cells surrounding the bone spicules.

activating missense mutation in exon 8 of the alpha chain of Gs is present in tissue from these various monostotic fibrous lesions.

METHODS

Four patients with monostotic fibrous dysplasia were studied. All of the patients presented for biopsy or curettage of an isolated bone lesion. One lesion was in the jaw, one in the tibia, one in the femur, and one in the pelvis. There were two men and two women. They ranged from 9 to 25 years of age at the time of surgery. A radiographic survey was performed on all the patients to determine if there were any additional lesions, and none were identified. None of the patients had *café au lait* spots or clinical evidence of an endocrinopathy. The patients were otherwise healthy and there was no family history of McCune-Albright syndrome or endocrinopathy.

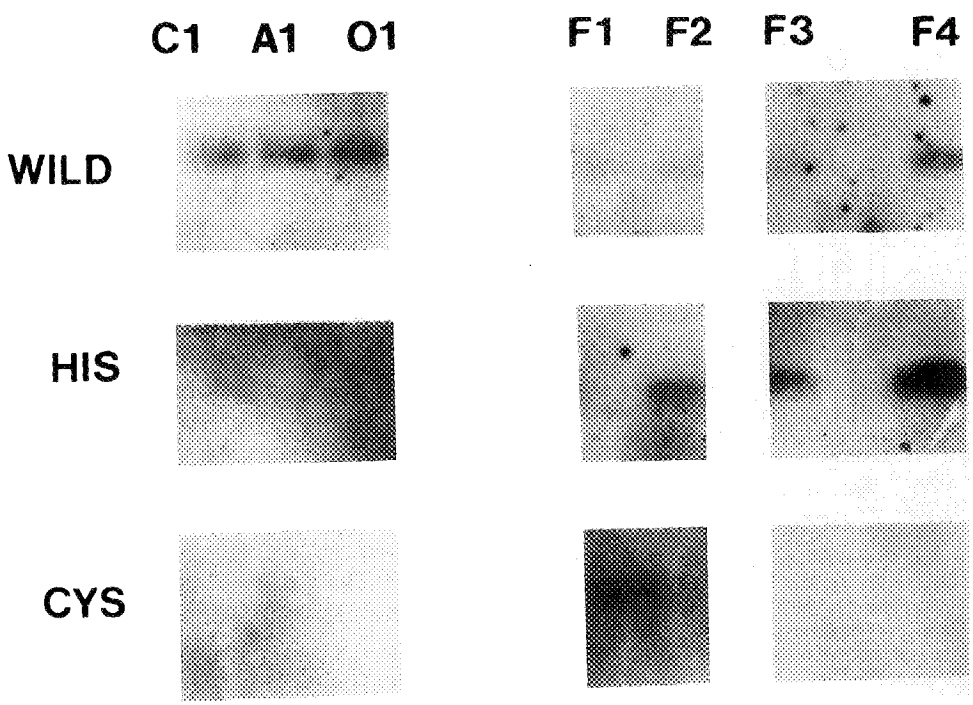

FIG. 2. Southern blot with use of oligonucleotide probes to detect point mutations at codon 201, resulting in a substitution of cysteine (TGT, labeled CYS) or histidine (CAT, labeled HIS) for the wild-type arginine (CGT, labeled WILD). Normal bone from the patient with fibrous dysplasia (lane C), aggressive fibromatosis (lane A), and osteofibrous dysplasia (lane O) all hybridized primarily to the wild-type probe. The four cases of fibrous dysplasia (lanes F1-F4) all hybridized to both the wild-type and a mutant probe, with one (F1) hybridizing to the cysteine probe and the other cases (F2-F4) hybridizing to the histidine probe.

One patient with osteofibrous dysplasia was studied. This patient was operated on at skeletal maturity for deformity of the tibia. A skeletal survey showed no evidence of other bone lesions.

Four patients with an aggressive fibromatosis involving bone were studied. Three had soft-tissue lesions that most likely originated outside the bone, and one had a lesion clearly originating within the bone itself. None of the patients showed evidence of other bone lesions on a skeletal survey or bone scan.

Additional tissue used in this investigation consisted of normal fascia from the patient with the aggressive fibromatosis arising within bone, normal bone from the patient with femoral fibrous dysplasia, normal fascia from the patient with fibrous dysplasia of the pelvis, normal bone from two additional subjects, and normal fascial tissue from three additional subjects.

All of the patients were operated on before undergoing any adjuvant therapies. The tissue removed from the lesions was divided and a portion was decalcified when necessary, fixed in formalin, and embedded in paraffin for routine histologic examination. The remainder of the tissue was snap frozen and stored in liquid nitrogen vapor. DNA was extracted from the fresh-frozen tissues utilizing proteinase K digestion. gDNA from exon 8 of Gs was amplified with use of the polymerase chain reaction with oligonucleotide primers located within the introns flanking the exon (12) for 40 cycles with a 55°C annealing temperature. The resultant amplicons were subjected to electrophoresis on an agarose gel, stained with ethidium bromide, and examined under ultraviolet light to ensure that an amplicon of appropriate base-pair length was amplified. The electrophoresed amplicons were transferred to a nylon membrane, and Southern blot analysis was performed with three oligonucleotide probes to screen the endocrine tissue for mutations. One probe identified wild-type Gs protein (R201), a second probe identified a single base-pair mutation that results in a substitution of histidine for arginine (R201H), and the third probe identified a single base-pair substitution that encodes for cysteine (R201C) (8). The probes were end-labeled with digoxigenin dd-UTP using a standard protocol (Boehringer Mannheim, Indianapolis, IN, U.S.A.). Southern blotting was performed with hybridization carried out overnight at 25°C in a buffer solution containing 50% formamide. The blot was exposed to Hyper film NP (Amersham, Arlington Heights, IL, U.S.A.) for 15 minutes without an intensifier. Each probe was hybridized separately, with the membranes stripped between each hybridization.

Additional polymerase chain reaction amplicons were used for sequencing. The amplicons were purified using the QIAquick polymerase chain reaction purification column (Quiagen, Chatsworth, CA, U.S.A.). Sequencing was performed with an automatic sequencer based on the polymerase chain reaction, using standard technique. Sequencing was performed in both forward and reverse directions, utilizing the upstream and downstream primers from the polymerase chain reaction.

RESULTS

Southern blot analysis showed that the normal bone from the patient with fibrous dysplasia of the femur, normal bone from the additional subjects, fascia from the patient with the aggressive fibromatosis, and fascia from the additional subjects all hybridized strongly to the wild-type probe (R201). Tissue from the monostotic fibrous dysplasia lesions all showed hybridization to the wild-type probe. However, in three of these lesions there was also strong hybridization to the R201H probe, detecting a histidine substitution, and the other lesion demonstrated strong hybridization to the R201C probe, detecting a cysteine substitution. There was very weak hybridization to the alternate mutant probe in all four cases. The one case of os-

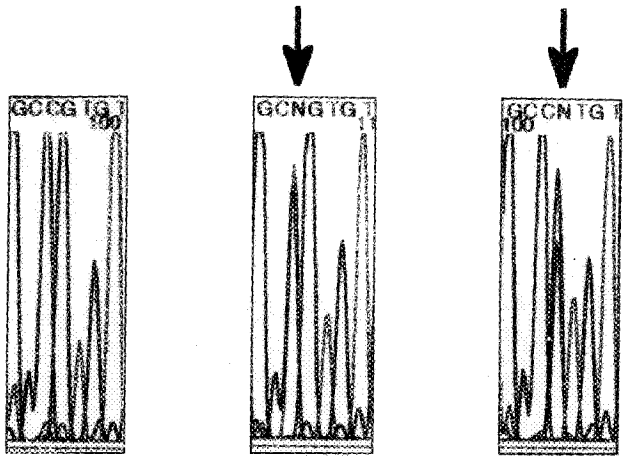

FIG. 3. Sequencing data that confirm the results of the Southern blot. The data shown are for normal bone from the patient with fibrous dysplasia of the femur (C on Fig. 2) and for the lesions of two patients with fibrous dysplasia (F1 and F2 on Fig. 2). The arrows point to the mutations, where two peaks (mutant and wild type) are identified. Sample 1 (F1) shows a substitution of TGT (cysteine) for wild-type CGT (arginine) at codon 201. Sample 2 shows a substitution of CAT (histidine) for CGT. A double peak is present in both cases, indicating either a herterozygous mutation or the presence of the wild-type sequence in stromal cells. The computer reading of the nucleotide sequence, therefore, is indeterminate, or "N."

teofibrous dysplasia and the four cases of aggressive fibromatosis all hybridized most strongly to the wild-type probe (Fig. 2).

Sequencing of exon 8 verified the results of Southern blot analysis. An identical sequence was obtained in forward and backward directions. Only the wild-type sequence was detected in the normal tissues, the aggressive fibromatoses, and the osteofibrous dysplasia. A mixture of wild-type and mutant sequences was identified in all four of the monostotic fibrous dysplasia cases (Fig. 3).

DISCUSSION

Monostotic fibrous dysplasia, unlike the endocrinopathies in which activating Gs protein mutations are identified, has only a local effect in bone. Adenylyl cyclase is therefore expected to result in a local effect, perhaps mediated by increased production of paracrine factors. Several growth factors are expressed by tissue from monostotic fibrous dysplasia: platelet-derived growth factor, insulin-like growth factor, and the bone morphogenetic proteins. The mutation may be responsible for increased expression of these factors, with resultant paracrine and autocrine effects on local cells.

Alternatively, the mutation may alter the normal development of a pluripotential mesenchymal cell that was destined to become a normal bone cell, perhaps arresting it at an intermediate stage. A third possibility is that this mutation is secondary to a preceding genetic or phenotypic event, such as a defect in a DNA repair mechanism, allowing the mutation to take place in this location. Activating Gs mutations can lead to autonomous cell proliferation in hormone-dependent endocrine cell lines (10), and perhaps in fibrous dysplasia the mutation contributes to the proliferation of the fibrocyte-like cells. There may be an increased risk of malignant transformation with a Gs activating mutation and, in fact, there is a higher incidence of local sarcoma occurrence with both monostotic and polyostotic fibrous dysplasia (11).

Osteofibrous dysplasia and aggressive fibromatosis did not demonstrate a Gs mutation in exon 8. This provides further evidence that osteofibrous dysplasia is not a variant of fibrous dysplasia but a distinct pathologic entity with a different molecular pathobiology. The similarities in cytologic morphology and gene expression of the aggressive fibromatoses and fibrous dysplasia are due to different molecular genetic mechanisms.

The anatomic distribution of the gene defect in McCune-Albright syndrome may be due to a mosaic distribution of abnormal cells during embryogenesis. In monostotic fibrous dysplasia, a similar gene defect is present in only one location in bone. Regardless of whether this lesion is part of a syndrome or isolated, there is likely a similar molecular pathobiology. The fibrous dysplasia lesions showed a mixture of mutant and wild-type Gs DNA. This may be due to a heterozygous mutation; however, a mixture of normal stromal cells within the bone lesion is also possible. As in McCune-Albright syndrome, both histidine and cysteine substitutions were identified in the monostotic lesions.

The case material for this study was limited to DNA; thus, one can only speculate about the presence of activated Gs protein, or mRNA containing the mutation. However, an activating missense mutation in Gs would be expected to behave much the same in bone as in other cellular locations. Further study of fresh tissue is needed to determine the protein function.

The occurrence of this mutation in fibrous dysplasia may aid in the pathologic diagnosis of fibrous dysplasia. It may be especially helpful in differentiating osteofibrous dysplasia from fibrous dysplasia in difficult cases. However, it is necessary to obtain fresh tissue, as decalcification usually makes it impossible to extract intact DNA for analysis. The best treatment of fibrous dysplasia is often problematic. Excision of this benign lesion frequently results in recurrence. The lesion may result in deformity or pathological fracture of the bone, both of which are difficult to treat without extensive surgical procedures. A pharmacologic treatment modality or one based on gene therapy

could result in a significant improvement in patient outcome. Perhaps such a treatment, based on G protein manipulation or cAMP regulation, can be developed for use in difficult cases of monostotic fibrous dysplasia.

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